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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

99307152.1

PRIORITY DOCUMENT
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Im Auftrag

For the President of the European Patent Office

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Uniform Molecular Weight Polymers

Introduction.

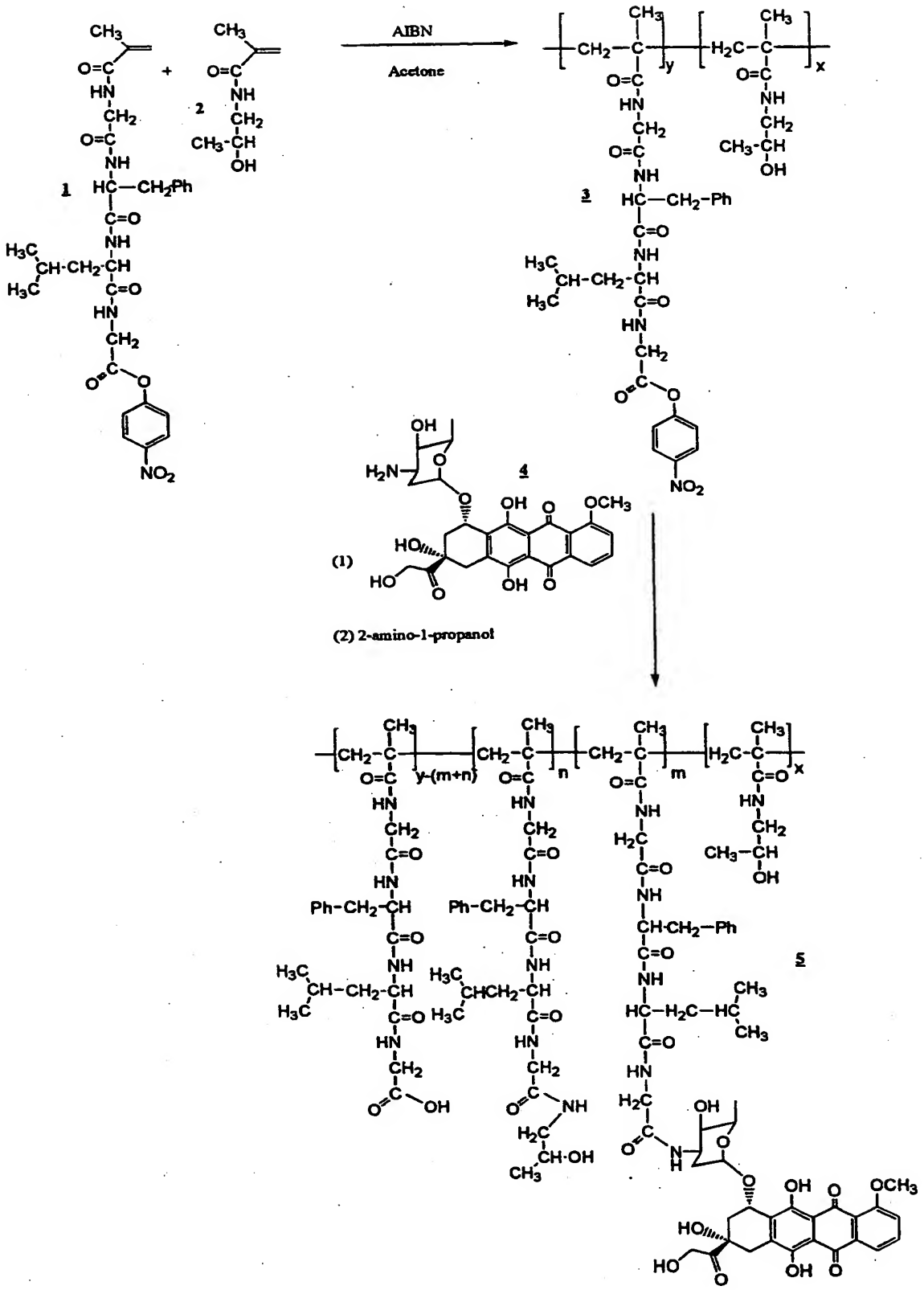
Covalent conjugation of a drug to a soluble, biocompatible polymer can exhibit improved efficacy compared to the free, unconjugated drug. Together the three main parts of a polymer-drug conjugate: (1) polymer, (2) linker and (3) conjugated drug produce a distinct profile of pharmacological, pharmacokinetic and physicochemical properties typical of polymer-drug conjugates. The polymer is not a mere carrier for the pharmacologically active drug. The properties of the polymer are directly responsible for defining the circulation half life, rate of cellular uptake, minimising toxicity of potent cytotoxic drugs, and imparting favourable physicochemical properties (e.g. increasing solubility of lipophilic drugs).

Polymers and polymer-drug conjugates currently used for medical applications are, from the perspective of regulatory agencies (e.g. Medicines Control Agency, FDA) not structurally defined. Many of these molecules and their conjugates exhibit broad molecular weight distribution and random co-monomer and/or conjugate incorporation. Future development of physiologically soluble polymers used in the development of polymer-drug conjugates (i.e. polymer therapeutics) requires that defined polymer structures be prepared (i.e. narrow molecular weight distribution and defined incorporation of drug conjugating pendent chains that have been conjugated with drug). This will ensure that more defined and optimised structures (e.g. polymers and conjugates) are carried forward in the development process.

Copolymers of N-(2-hydroxypropyl) methacrylamide (HPMA) have been extensively studied for the conjugation of cytotoxic drugs for cancer chemotherapy *[1-3]*. The homopolymer of HPMA is soluble in biological fluids, readily excreted at molecular weights of less than 40,000 Da *[4]*, is non-toxic up to 30 g/kg, does not bind blood proteins *[5]*, and is not immunogenic *[6-9]*. Like poly(ethylene glycol) (PEG) which is generally recognised as safe (GRAS), HPMA copolymers are biocompatible. Since HPMA copolymers are hydrophilic, solublisation of hydrophobic drugs is possible, e.g. doxorubicin and paclitaxel *[10-13]*. Four HPMA copolymer conjugates have progressed into Phase I/II trial *[14-18]*. An example is the HPMA copolymer doxorubicin conjugate known as PK1 which is currently undergoing Phase II evaluation in the UK. In the Phase I studies the HPMA copolymer-Gly-Phe-Leu-Gly-doxorubicin conjugate displayed greatly reduced toxicity compared to free doxorubicin and showed evidence of activity in chemotherapy refractory patients *[19-21]*. The maximum tolerated dose (MTD) of PK1 was 320 mg/m^2 (doxorubicin equivalent) which is 4-5 times higher than the usual clinical dose of free doxorubicin. There was no evidence of conjugate related

cardiotoxicity (despite individual cumulative doses of up to 1680 mg/m^2 doxorubicin-equivalent), and the dose limiting toxicity was bone marrow suppression. No polymer-related toxicity was observed. Other promising preclinical polymer-drug conjugates *[22,23]* may soon be clinically evaluated as anticancer compounds. The concept entailing the appropriate conjugation of a drug to a polymer for treatment of cancer is tangible, been proven viable in a clinical environment and is gaining widespread momentum.

Currently HPMA copolymer-drug conjugates (e.g. PK1) are prepared by a polymer analogous reaction of a low molecular weight drug (e.g. doxorubicin **4**) and an active ester peptidic polymeric precursor **3** (Scheme 1) *[9,24-28]*. This strategy which has been used to optimise and prepare polymer-drug conjugates to strict industrial criteria on a scale necessary for clinical evaluation has the significant advantage that a common polymeric intermediate (e.g. **3**) can be prepared and used to prepare polymer-drug conjugates with different drugs all having the same molecular weight characteristics *[29]*. However it is not possible to use one polymer conjugate **3** to vary the drug loading or pendent chain structure. Additional precursors analogous to **3** must be prepared. Also many conjugates have been prepared using **3**, however the competitive hydrolysis of the *p*-nitrophenol ester actually produces conjugates **5** (the actual structure of PK1) that have peptidic pendent chains terminated with either drug, carboxylate, or aminopropanol (Scheme 1) *[30,31]*. Additionally, the free radical polymerisation gives **3** as a random copolymer typically with a polydisperisity (PD)>2.5. Incorporation of different amounts of **2** or monomers with different peptides requires that the polymerisation conditions be optimised to obtain reproducible molecular weights under the renal threshold.


AIBN
Acetone
1
2
3
4
5
(1)
(2) 2-amino-1-propanol

Scheme 1.

In principal, it is possible to alter drug loading by varying the stoichiometry of **4**, but the final conjugate **5** will still contain mixtures of the peptidic side pendent chains statistically distributed over a broad molecular weight distribution. Lysosomal degradation of non-drug conjugated pendent chains will compete with degradation of the drug conjugated pendent chains. This competition complicates the pharmacology and pharmacokinetics for polymer-drug conjugates. From the viewpoint of drug regulatory authorities, the polymerisation and the conjugation processes depicted in Scheme 1 result in final polymer-drug conjugates (e.g. **5**) that are extremely varied in structure and thus difficult to regulate as a medicinal agent.

During preclinical and clinical evaluation of any drug, the identification and pharmacological/pharmacokinetic characterisation of all chemical species related to the candidate drug (e.g. racemates, manufacturing impurities, metabolites, etc.) must be elucidated. Polymer-drug conjugates tend to be non-uniform with respect to molecular weight of the polymer and the location and number of drug conjugating pendent chains along the polymer mainchain. Polymer therapeutics must be rigorously characterised with respect to their molecular weight and polydispersity since biodistribution and pharmacological activity are known to be molecular weight-dependent. For example, blood circulation half-life *[32]*, renal clearance, deposition in organs *[33]*, rates of endocytic uptake *[34,35]* and biological activity can depend on polymer molecular weight characteristics *[36-41]*. While HPMA copolymers currently undergoing clinical evaluation exhibit increased efficacy and a considerable amount of the biological rationale for polymer-drug conjugates has been elucidated, the fact is these therapeutic compounds exist as broad statistical distributions in respect to molecular weight and structure of conjugation pendent chains. This is problematic from a regulatory stand-point, especially for the repeat dosing of polymer-drug conjugates in the treatment of chronic conditions. For example, it would be difficult to ascertain if long term effects were due to low or high molecular weight species in a polydisperse therapeutic conjugate.

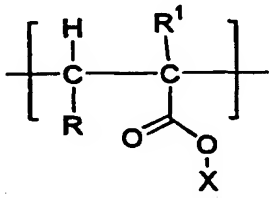
It is evident that mixtures of structures (e.g. **5**) are not optimal (Scheme 1). Structure variation due to the broad distribution of polymer molecular weights and random incorporation of drug conjugating pendent chains must be minimised for more widespread development of polymer-drug conjugates. This invention describes a process for preparing specifically polymer-drug conjugates that have a narrow distribution of polymer molecular weights. By an extension of the inventive process it is possible to prepare narrow molecular weight block copolymer-drug

conjugates. In this way it is possible to prepare polymer-drug conjugates that do not have completely random incorporation of drug conjugating pendent chains. A need therefore exists to prepare uniform polymer-drug conjugates in a practical way. Two broad needs exist: (1) preparation of polymers of uniform molecular weights (i.e. preparation of monodispersed polymers) and (2) controlling the location of the drug conjugating pendent chains along the polymer mainchain (e.g. preparation of block copolymers).

It is possible to prepare polymer-drug conjugates with narrow molecular weight distribution of PD<1.2 using controlled radical polymerisation processes. Typically only anionic processes can be used to prepare polymers with such narrow molecular weight distributions. The experimental conditions for these types of reactions are difficult to control and require the complete exclusion of oxygen, moisture, and exchangeable protons. In contrast CRP processes (e.g. (RAFT (Reversible Fragmentation Chain Transfer Polymerisation)) *[42]*, ATP (Atom Transfer Polymerization *)[43,44]* and NMP *[45,46]*) are currently being widely used to prepare polymers with narrow molecular weight distribution and defined structure by essentially free radical processes.

Summary of the Invention

One embodiment of the present invention provides a polymer comprising the unit (I)



(I)

where R is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl or any one of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl substituted with a heteroatom within, or attached to, the carbon backbone; R^1 is selected from the group consisting of C_1-C_6 alkyl groups; X is an carboxylate activating group and wherein the polymer has a polydispersity of less than 1.2 and a molecular weight (Mw) of less than 50,000.

The carboxylate activating group X is generally selected from the group consisting of N-succinimidyl, pentachlorophenyl, pentafluorophenyl, para-nitrophenyl, dinitrophenyl, N-phthalimido, N-norbornyl, cyanomethyl, pyridyl, trichlorotriazine, 5-chloroquinilino, and imidazole. Preferably X is an N-succinimidyl or imidazole moiety.

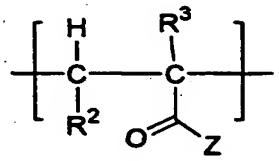
Preferably R is selected from the group consisting of hydrogen,

C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 aralkyl and C_1-C_6 alkaryl, C_1-C_6 alkanoyl, C_1-C_6 alkylamido and C_1-C_6 alkylimido. Most preferably R is selected from hydrogen or methyl.

Preferably R^1 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl or isomers thereof. Most preferably R^1 is selected from hydrogen or methyl.

The polymer of the present invention may be a homopolymer incorporating unit (I), or may be a copolymer or block copolymer incorporating other polymeric, oligomeric or monomeric units. For example, further polymeric units incorporated in the polymer may comprise acrylic polymers, alkylene polymers, urethane polymers, amide polymers, polypeptides, polysaccharides and ester polymers. Preferably, where the polymer is a heteropolymer, additional polymeric components comprise polyethylene glycol, polyaconitic acid or polyesters. The molecular weight of the polymer should ideally be less than 50,000 in order that the renal threshold is not exceeded. Preferably the molecular weight of the polymer is in the range of 5-40,000, more preferably 25,000-40,000.

Another embodiment of the present invention is a polymer comprising the unit (II)



(II)

wherein R^2 is hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl, R^3 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl and isomers thereof, Z is a pendent group selected from the group consisting of $N(R^4)_2$, SR^5 and OR^6, wherein NR^4 is an aminoacyl group or N(oligopeptidyl group; R^6 and R^7 are selected from the group consisting of hydrogen, C_1C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy and C_1-C_{12} hydroxyalkyl, and may contain one or more cleavable bonds and may be covalently linked to a bioactive agent, wherein the polymer has a polydispersity of less than 1.2 and a molecular weight (Mw) of less than 50,000.

Preferably R^2 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 aralkyl and C_1-C_6 alkaryl, C_1-C_6 alkanoyl, C_1-C_6 alkylamido and C_1-C_6 alkylimido.

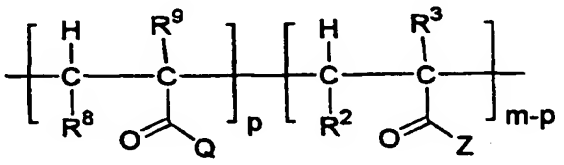
Preferably R^3 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl or isomers thereof. Most preferably R^2 is hydrogen and R^3 is methyl.

Z may comprise one or more aminoacyl groups, preferably 2-6 aminoacyl groups, most preferably 4 aminoacyl groups. In a particularly preferred embodiment group Z comprises a glycine-leucine-phenylalanine-glycine linkage. The aminoacyl linkage is most preferably a peptide linkage capable of being cleaved by preselected cellular enzymes, for instance, those found in liposome of cancerous cells.

In another preferred embodiment group Z comprises a cis-aconityl group. This group is designed to remain stable in plasma at neutral pH (~7.4), but degrade intracellularly by hydrolysis in the more acidic environment of the endosome or liposome (~pH 5.5-6.5).

The pendent chain Z may additionally be covalently bound to a bioactive agent. Preferred bioactive agents are anti-cancer agents such as doxorubicin, dornomycin, taxol or paclaxitol.

A further preferred polymer of the present invention has the structure (III)

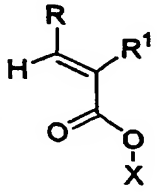


(III)

wherein R^8 and R^9 are selected from the same groups as R^2 and R^3 respectively, Q is a solubilising groups selected from the group consisting of C_1C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12} hydroxyalkyl, C_1-C_{12} alkylamido, C_1-C_{12} alkylimido, C_1-C_{12} alkanoyl, and wherein p is an integer of less than 500.

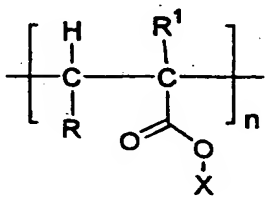
Preferably Q comprises an amine group, preferably a C_1-C_{12} hydroxyalkylamino group, most preferably a 2-hydroxypropylamino moiety. This group is designed to be a solubilising group for the polymer in aqueous solutions. Generally the polymer of the present invention is a water soluble polyacrylate ester.

In a further embodiment, the present invention provides a process for the production of a polymer, comprising the polymerisation of a compound (IV)



(IV)

wherein R is selected from hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl, is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl and isomers thereof; X is a carboxylate activating group; wherein the polymerisation is a Controlled Radical Polymerisation, selected from the group consisting of Reversible Addition Chain Transfer Polymerisation, Atom Transfer Radical Polymerisation and Nitroxide Mediated Polymerisation, to produce a polymer comprising the unit (I)



(I)

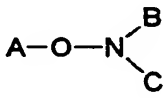
wherein R and R^1 are as defined hereinbefore and n is an integer of 1 to 500.

Preferably R is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 aralkyl and C_1-C_6 alkaryl, C_1-C_6 alkanoyl, C_1-C_6 alkylamido and C_1-C_6 alkylimido.

Preferably R^1 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl or isomers thereof.

Where the polymerization is carried out by atom transfer radical polymerization, a suitable radical initiator is utilised. Such initiators commonly comprise a Cu(I) complex. Such complexes are usually Cu(I)Br complexes, complexed by a chelating ligand. Typical initiators are Cu(I)Br $(Bipy)_2$ and Cu(I)Br (Pentamethyl diethylene). The reaction should take place in the presence of a suitable solvent. Such solvents are generally aprotic solvents, for example tetrahydrofuran, acetonitrile, dimethylformamide, acetone, dimethylsulphoxide, methylformamide and sulpholane.

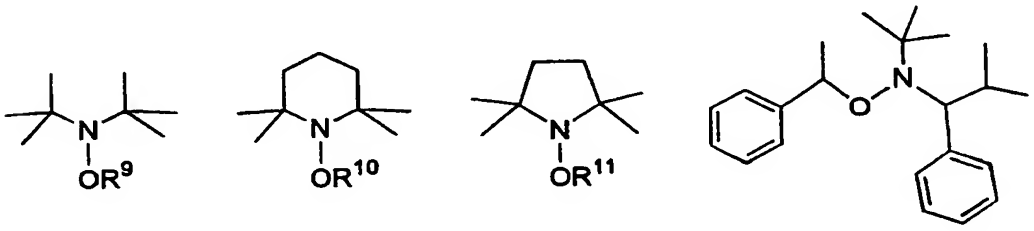
Alternatively the polymerization may take place via Nitroxide Mediated Polymerization. Again, a suitable Nitroxide Mediated Polymerization initiator is required. Such an initiator generally has the structure



wherein A is selected from the group consisting of C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} hydroxyalkyl, B and C are individually selected from the group consisting of C_1C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} hydroxyalkyl, and may together with N form a C_1-C_{12} heterocyclic group and which may contain a heteroatom selected from nitrogen, sulfur, oxygen and phosphorus.

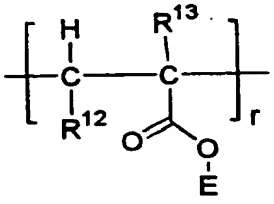
Preferably A is selected from the group consisting of methyl, ethyl, propyl, butyl, pentyl, hexyl, benzyl, methylbenzene, ethyl benzene, propylbenzene or isomers thereof. B and C should generally be sterically crowding the groups capable of stabilising a nitroxide radical. Such groups are generally selected from the group consisting of isopropyl, isobutyl, secbutyl, tert-butyl, isopentyl, sec-pentyl, tert-pentyl, adamantyl, methylbenzene, ethyl benzene, propylbenzene or isomers thereof.

Common initiators have these structures outlined below



wherein R^9 to R^{11} are selected from the group consisting of C^1-C^{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12} hydroxyalkyl, C_1-C_{12} alkylamido, C_1-C_{12} alkylimido, C_1-C_{12} alkanoyl.

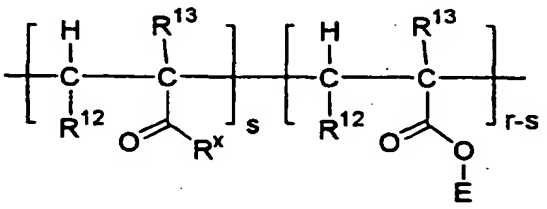
A further embodiment of the present invention provides a process for the production of a polymer, comprising the reaction of a polymer having the formula (VI)



(VI)

wherein R^{12} is a group selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl and C_1-C_{18} alkaryl groups; R^{13} is selected from the group consisting of C_1-C_6 alkyl groups; E is a carboxylate activating group and r is an integer of 5 to 500; with a reagent HR^x, wherein R^x is selected from the group consisting of $N(R^{14})_2$, SR^{15}, OR^{16}, wherein R^{14} to R^{17} are individually selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12}

hydroxyalkyl, C_1-C_{12} alkylamido, C_1-C_{12} alkylimido, C_1-C_{12} alkanoyl, and may contain one or more cleavable bonds, to form a derivatised polymer having the structure (VII)



(VII)

wherein $1 \leq s \leq r$.

R^{12} is preferably selected from the group consisting of hydrogen, methyl, ethyl and propyl, R^{13} is selected from the group consisting of hydrogen, methyl, ethyl and propyl and preferably R^{12} is hydrogen and R^{13} are methyl.

E is selected from the group consisting of N-succinimidyl, pentachlorophenyl, pentafluorophenyl, para-nitrophenyl, dinitrophenyl, N-phthalimido, N-norbornyl, cyanomethyl, pyridyl, trichlorotriazine, 5-chloroquinilino, and imidazole, preferably N-succinimidyl or imidazole, most preferably N-succinimidyl.

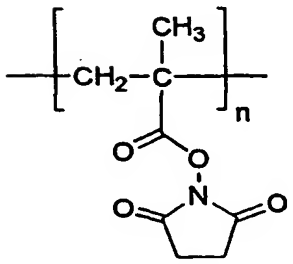
Preferably HR^x is NR^{14}.

HR^x is generally a nucleophilic reagent capable of displacing E-O, to form a covalent bond with the acyl group attached to CR^{13}. Preferably HR^x comprises a primary or secondary amine group. Most preferably HR^x comprises a cleavable bond such as a aminoacyl linkage or a cis-aconityl linkage as described hereinbefore. Generally D is covalently attached to a bioactive agent prior to reaction with (VI) subsequent to the production of a polymer having the structure (VII), an additional step of quenching the polymer may take place. This involves reacting the previously unreacted groups E with a quenching group. This group preferably comprises an amine moiety and is generally selected to be a solubilising group for the polymer. Such a quenching compound is, for example, hydroxypropylamine.

Detailed Description of the Invention

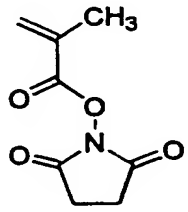
The present invention provides a polymer having a polydispersity of less than 1.2. The polymer is preferably an activated polyacrylate ester that is prepared by Controlled Radical Polymerization. These polymers are designed to be derivitisable and may be used to form polymer-drug conjugates having improved biological profile.

A particularly preferred polymer of the present invention comprises the structure (X)



(X)

The activating moiety is an N-succinimidyl group. This particular group has been found to be particularly stable in solution and resists spontaneous hydrolysis. This polymer is produced by Atom Transfer Polymerization using a Cu(I)Br(pentamethyldiethylene) initiator. The polymerization involved the reaction of a monomer (IX)



(IX)

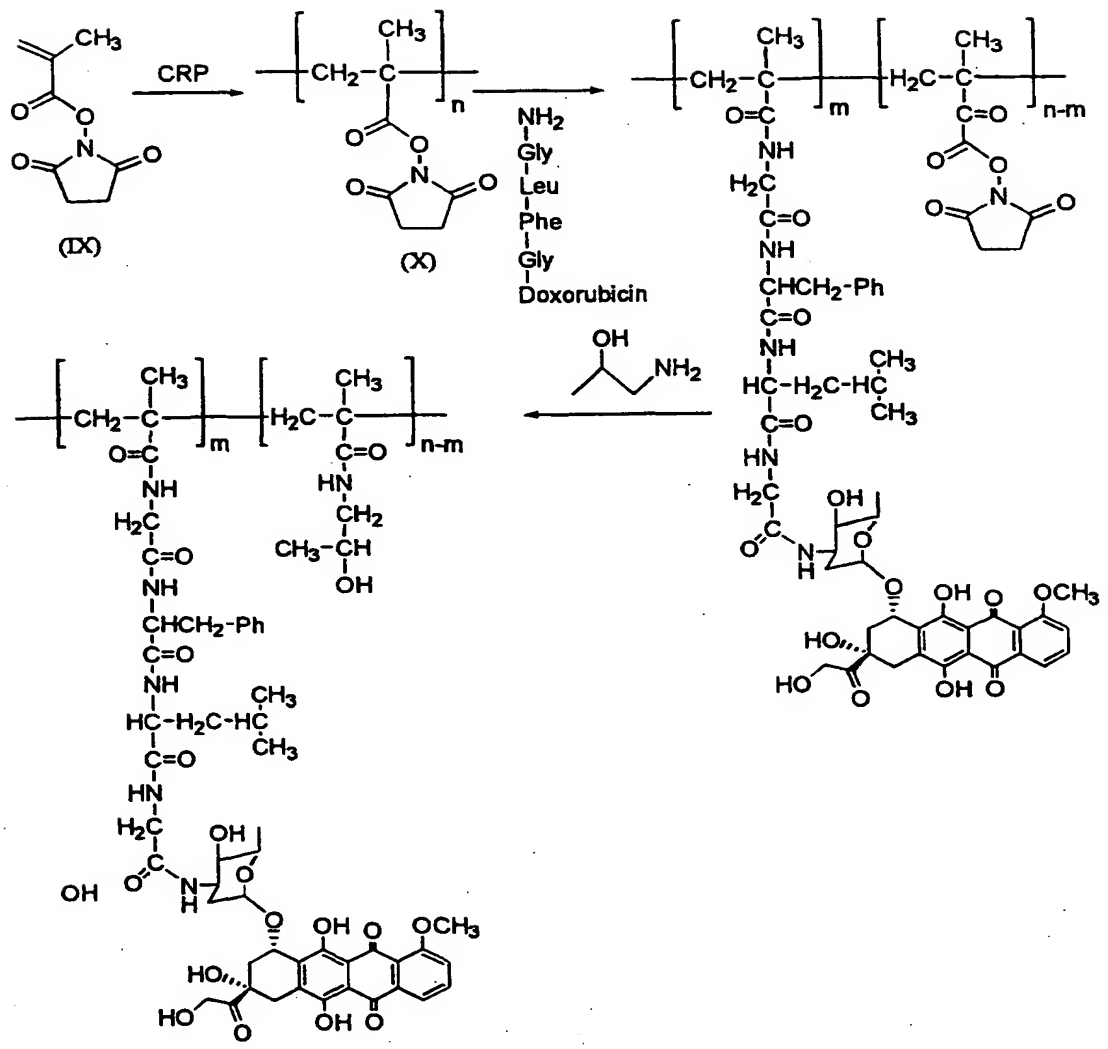
with a suitable aprotic solvent. In a preferred embodiment the solvent is tetrahydrofuran. The reaction is preferably carried out under a nitrogen atmosphere and at a temperature of 30-150°C. A preferred temperature range is 50-80°C, most preferably 70°C.

The polymer comprising the unit (X) may subsequently be derivatised. The carboxyl activating group may be substituted by a suitable nucleophilic reagent. In order to form polymer drug conjugates it is preferable to derivatise unit (X) with a pendant moiety. Such a moiety could comprise a aminoacyl linkage or a hydrolytically labile linkage as defined hereinbefore. Such a linkage can degrade when entering the lysosome of a diseased cell, thus releasing a drug or drug precursor directly to the target site.

Preferably a pendent moiety comprises a Gly-Leu-Phe-Gly linkage or a cis aconityl linkage. Such a pendent linkage may be covalently attached to a drug prior to polymer derivitisation or may be capable of being derivatised subsequent of attachment of the pendent moiety to the polymer backbone.

In a preferred embodiment the polymer comprising the unit (X) is reacted with less than 1 equivalent of a pendent group, thus only substituting a pre-specified number of N-succinimidyl moieties. This allows a second,

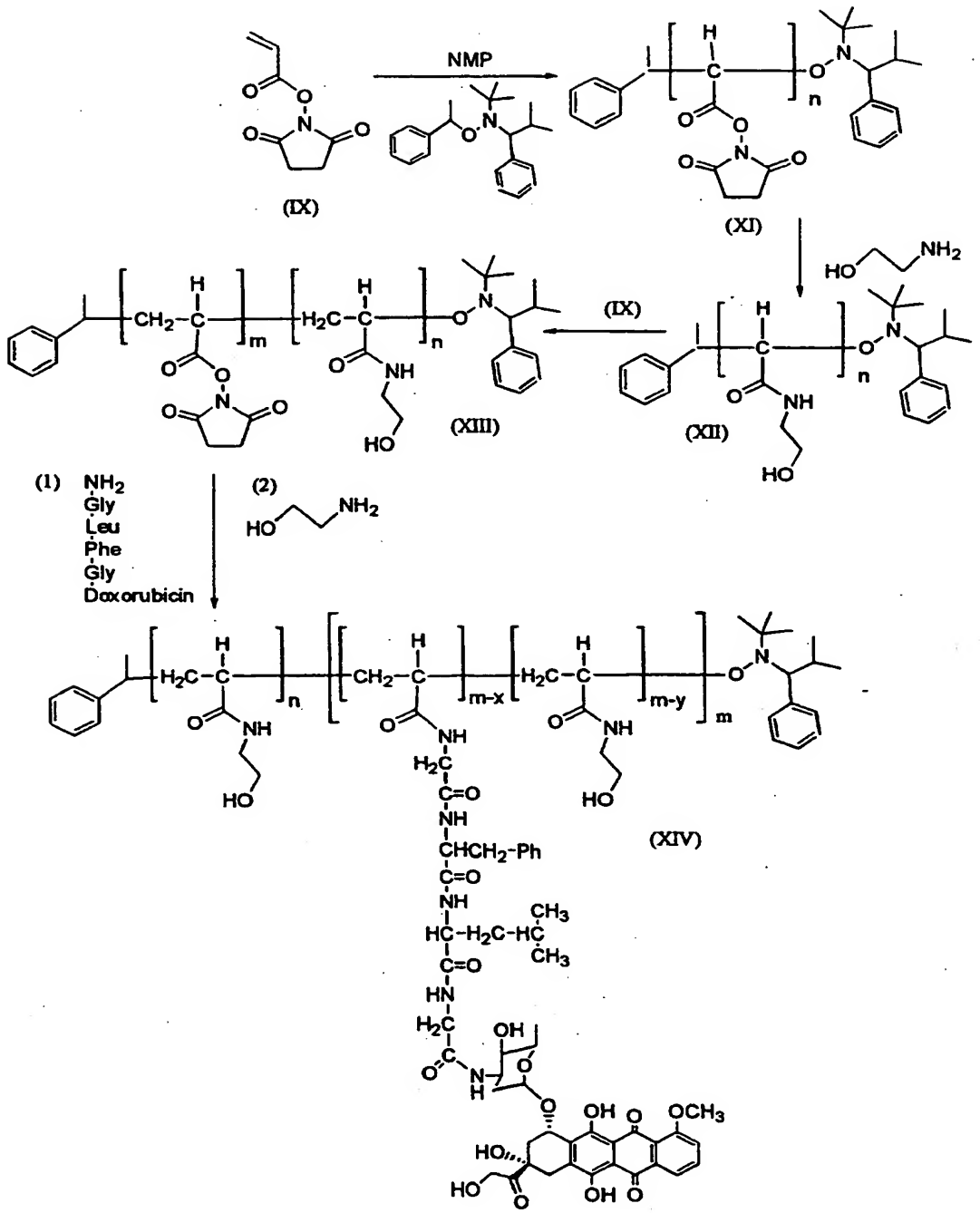
quenching step, which substitutes the remaining N-succinimidyl groups with a solubilising group. Such a group aids in the solubilisation of the polymer in aqueous solutions such as biological fluids. A preferred quenching agent should comprise an amine, for example 2-hydroxypropylamine. An overview of a preferred reaction process is provided in scheme 2 below. In this particular example, the drug doxorubicin is attached to the polymer via a GLFG linkage.



Scheme 2

n is an integer in the range of 1 to 500 and m is the number equivalent of pendent moieties reacted with the activated polymer.

CRP processes are known to result in the presence of dormant initiating moieties at the chain ends of linear polymers. In particular the use of nitroxide mediated radical polymerization may be used to prepare narrow molecular weight distributed block copolymers. This allows more defined introduction of drug conjugating pendent chains in the polymer. Outlined in Scheme 3 is an example of this approach to prepare a block copolymer precursor using the CRP process known as nitroxide mediated polymerization (NMP).



wherein x and y are the number equivalent of the pendent moiety and quenching group respectively.

Thus, polymeric precursors (XI) and (XIII) are designed to be used as polymeric precursors for polymer analogous reactions that are driven to completion to prepare conjugates with narrow molecular weight distributions and with differing *m* and *n* repeat structure. Drug conjugation would be localized only in the n repeat structure. Again it is possible to vary the solubilising pendent chain and the drug conjugating pendent chain starting

from the polymeric precursor (XI). Defining the location of the drug conjugating pendent chains is necessary to develop more defined polymer-drug conjugates. The extent and location of drug loading and its influence on polymer solution properties is an important, and yet poorly understood phenomenon and will have a fundamental effect on the *in vivo* properties of therapeutic polymer-conjugates. Thus, this approach will find utility also in the development and optimization of polymer-drug conjugates.

Examples

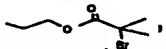
Copper(I) bromide, pentamethyldiethylene ligand, an initiator having the structure  , and monomer (IX) were added to THF solvent is a glass flask. The resulting solution was purged with nitrogen to remove oxygen. The flask was sealed and placed in an oil bath at 70°C for 24 Hr. Samples were prepared for gel permeation chromatography by passing through a neutral aluminium oxide column to remove copper components. Analysis reveals the production of a polymer with a molecular weight of 20,000. A sample of this activated ester homopolymer was quenched with 1-aminopropanol, to give a water soluble polymer whose ^{1}H NMR spectrum was consistent with that of poly(HPMA). Figure 1 compares the gel permeation chromatograms of HPMA homopolymer prepared from conventional free radical polymerization with that of 1-aminopropanol quenched poly(methacryloylsuccinimide) prepared using atom transfer radical polymerization.

Figure 1 shows that the broad molecular weight distribution associated with conventional free radical polymerization can be greatly improved using ATP.

References

(1) Duncan, R.; Dimitrijevic, S.; Evagorou, E. *STP Pharma* **1996**, 237-263.
(2) Putnam, D.; Kopecek, J. *Adv.Polym.Sci.* **1995**, *122*, 55-123.
(3) Duncan, R. *Anti-Cancer Drugs* **1992**, *3*, 175-210.
(4) Seymour, L.; Duncan, R.; Strohalm, J.; Kopecek, J. *J. Biomed. Mater. Res.* **1987**, *21*, 1341-1358.
(5) Chytry, V.; Kopecek, J.; Sikk, P.; Sinijarv, R.; Aaviksaar, A. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 11-15.
(6) Rihova, B.; Ulbrich, K.; Kopecek, J.; Mancal, P. *Folia Microbiol.* **1983**, *28*, 217-297.
(7) Rihova, B.; Kopecek, J.; Ulbrich, K.; Chytry, V. *Makromol. Chem. Suppl.* **1985**, *9*, 13-24.
(8) Rihova, B.; Riha, I. *CRC Crit. Rev. Therap. Drug Carrier Sys.* **1985**, *1*, 311-374.
(9) Rihova, B.; Ulbrich, K.; Strohalm, J.; Vetvicka, V.; Bilej, M.; Duncan, R.; Kopecek, J. *Biomaterials* **1989**, *10*, 335-342.
(10) Pesenti, E.; Franzetti, C.; Biasoli, G.; Ciomei, M.; Paston, W.; Marsiglio, A.; Stegnjaich, S.; Mongelli, N.; Grandi, M. *Proc. Am. Assoc. Cancer Res.* **1995**, 1824.
(11) Mongelli, N.; Pesenti, E.; Suarato, A.; Biasoli, G. *US Patent, 5,362,831* **1994**.
(12) Huniunk, W. B.; Terwogt, J. M.; Dubbelman, R.; Valkenet, L.; Zurlo, M.; Schellens, J.; Beijnen, J. In *Proceedings of the 3rd Int'l. Symp. on Polymer Therapeutics* London, 1998, p 12.
(13) Mendichi, R.; Rizzo, V.; Gigli, M.; Razzano, G.; Angelucci, F.; Schieroni, A. *J. Liq. Chromatogr. Relat. Technol.* **1998**, *21*, 1295-1309.
(14) Duncan, R.; Seymour, L.; Scarlett, L.; Lloyd, J.; Rejmanova, P.; Kopecek, J. *Biochim. Biophys. Acta.* **1986**, *880*, 62-71.
(15) Seymour, L.; Ulbrich, K.; Strohalm, J.; Duncan, R. *Brit. J. Cancer* **1991**, *63*, 859-866.
(16) O'Hare, K.; Hume, I.; Scarlett, L.; Duncan, R. *Hepatology* **1989**, *10*, 207-214.
(17) Seymour, L.; Ferry, D.; Boivin, C.; Julyan, P.; Doran, J.; David, M.; Anderson, D.; Christodoulou, C.; Young, A.; Kerr, D.; Hesslewood, S. *Proc. Int'l. Symp. Recent Adv. Drug Del. Syst.* **1997**, *8*, 132-135.
(18) Kerr, D.; Seymour, L.; Boivin, C.; Julyan, P.; Doran, J.; David, M.; Anderson, D.; Christodoulou, C.; Daryani, S.; Young, A.; Hesslewood, S.;

Ferry, D. In *Proceedings of the 3rd Int'l. Symp. on Polymer Therapeutics* London, 1998, p 23.

(19) Vasey, P.; Twelves, C.; Kaye, S.; Wilson, P.; Morrison, R.; Duncan, R.; Thomson, A.; Hilditch, T.; Murray, T.; Burtles, S.; Cassidy, J. *Clin. Cancer Res.* **1999**, *5*, 83-94.

(20) Vasey, P.; Duncan, R.; Twelves, C.; Kaye, S.; Strolin-Benedetti, M.; Cassidy, J. *Annals of Oncology* **1996**, *7*, 97.

(21) Vasey, P.; Duncan, R.; Kaye, S.; Cassidy, J. *Eur. J. Cancer* **1995**, *31A*, S193.

(22) Venne, A.; Li, S.; Mandeville, R.; Kabanov, A.; Alakhov, V. *Cancer Res.* **1996**, *56*, 3626-3629.

(23) Batrakova, E.; Dorodnych, T.; Klinskii, E.; Kliushnenkova, E.; Shemchukova, O.; Goncharova, O.; Ajakov, S.; Alakhov, V.; Kabanov, A. *Br. J. Cancer* **1996**, *74*, 1545-1552.

(24) Kopecek, J.; Bazilova, H. *Eur. Polymer J.* **1973**, *9*, 7-14.

(25) Strohalm, J.; Kopecek, J. *Angew. Makromol. Chem.* **1978**, *70*, 109-118.

(26) Rejmanova, P.; Labsky, J.; Kopecek, J. *Makromol. Chem.* **1977**, *178*, 2159-2168.

(27) Kopecek, J. *Makromol. Chem.* **1977**, *178*, 2169-2183.

(28) Kopecek, J. *J. Cont. Rel.* **1990**, *11*, 279-290.

(29) Mendichi, R.; Rizzo, V.; Gigli, M.; Schieroni, A. G. *J. Liq. Chrom. and Rel. Technol.* **1996**, *19*, 1591-1605.

(30) Configliacchi, E.; Razzano, G.; Rizzo, V.; Vigevani, A. *J. Pharm. Biomed. Analysis* **1996**, *15*, 123-129.

(31) Pinciroli, V.; Rizzo, V.; Angelucci, F.; Tato, M.; Vigevani, A. *Magn. Reson. Chem.* **1997**, *35*, 2-8.

(32) Cartlidge, S.; Duncan, R.; Lloyd, J.; Kopeckova-Rejmanova, P.; Kopecek, J. *J Con. Rel.* **1986**, *4*, 253-264.

(33) Sprincl, L.; Exner, J.; Sterba, O.; Kopecek, J. *J. Biomed. Mater. Res.* **1976**, *10*, 953-963.

(34) Duncan, R.; Pratten, M.; Cable, H.; Ringsdorf, H.; Lloyd, J. *Biochem. J.* **1981**, *196*, 49-55.

(35) Cartlidge, S.; Duncan, R.; Lloyd, J.; Rejmanova, P.; Kopecek, J. *J. Cont. Rel.* **1986**, *3*, 55-66.

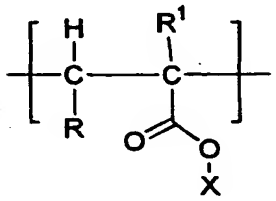
(36) Kaplan, A. In *Anionic Polymeric Drugs*; Donaruma, L., Ottenbrite, R., Vogl, O., Eds.; Wiley: New York, 1980, p 227-254.

(37) Ottenbrite, R.; Regelson, W.; Kaplan, A.; Carchman, R.; Morahan, P.; Munson, A. In *Polymeric Drugs*; Donaruma, L., Vogl, O., Eds.; Academic Press: New York, 1978, p 263-304.

(38) Butler, G. In *Anionic Polymeric Drugs*; Donaruma, L., Ottenbrite, R., Vogl, O., Eds.; Wiley: New York, 1980, p 49-142.
(39) Muck, K.; Rolly, H.; Burg, K. *Makromol. Chem.* **1977**, *178*, 2773.
(40) Muck, K.; Christ, O.; Keller, H. *Makromol. Chem.* **1977**, *178*, 2785.
(41) Seymour, L. *J. Bioact. Compat. Polymers* **1991**, *6*, 178-216.
(42) Chiefari, J.; Chong, Y.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T.; Mayadunne, R.; meijs, G.; Moad, C.; Moad, G.; Rizzardo, E.; Thang, S. *Macromolecules* **1998**, *31*, 5559-5562.
(43) Wang, J.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614.
(44) Wang, J.; Matyjaszewski, K. *Macromolecules* **1995**, *28*, 7901.
(45) Benoit, D.; Chaplinski, V.; Braslau, R.; Hawker, C. *J. Am. Chem. Soc.* **1999**, *121*, 3904-3920.
(46) Dao, j.; Benoit, D.; Hawker, C. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 2161-2167.
(47) Batz, H.; Franzmann, G.; Ringsdorf, H. *Angew. Chem. Internat. Edit.* **1972**, *11*, 1103-1104.
(48) Batz, H.; Franzmann, G.; Ringsdorf, H. *Makromol. Chem.* **1973**, *172*, 27-47.
(49) Batz, H.; Ringsdorf, H.; Ritter, H. *Makromol. Chem.* **1974**, *175*, 2229-2239.
(50) Ferruti, P.; Bettelli, A.; Fere, A. *Polymer* **1972**, *13*, 462-464.
(51) Nazarova, O.; Solovskij, M.; Panarin, E.; Denisov, V.; Kachaturov, A.; Koltsov, A.; Purkina, A. *Eur. Polym. J.* **1992**, *28*, 97-100.
(52) Takeuchi, S.; Oike, M.; Kowitz, C.; Shimasaki, C.; Hasegawa, K.; Kitano, H. *Makromol. Chem.* **1993**, *194*, 551-558.

CLAIMS

1. A polymer comprising the unit (I)



(I)

where R is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl or any one of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl substituted with a heteroatom within, or attached to, the carbon backbone; R^1 is selected from the group consisting of C_1-C_6 alkyl groups; X is an carboxylate activating group and wherein the polymer has a polydispersity of less than 1.2 and a molecular weight (Mw) of less than 50,000.

2. The polymer according to claim 1, wherein X is selected from the group consisting of N-succinimidyl, pentachlorophenyl, pentafluorophenyl, para-nitrophenyl, dinitrophenyl, N-phthalimido, N-norbornyl, cyanomethyl, pyridyl, trichlorotriazine, 5-chloroquinilino, and imidazole, preferably N-succinimidyl or imidazole, most preferably N-succinimidyl.

3. The polymer according to claim 1 or claim 2, wherein R is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 aralkyl and C_1-C_6 alkaryl, C_1-C_6 alkanoyl, C_1-C_6 alkylamido and C_1-C_6 alkylimido, preferably hydrogen of methyl.

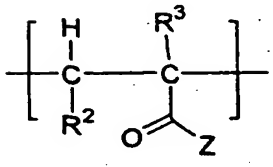
4. The polymer according to any preceding claim, wherein R^1 is hydrogen, methyl, ethyl, propyl, butyl, pentyl or isomers thereof, preferably hydrogen or methyl.

5. The polymer according to any preceding claim, wherein the molecular weight (Mw) is in the range 5000-4000, preferably 25,000 - 40,000.

6. The polymer according to any preceding claim, wherein R is hydrogen, R^1 is methyl.

7. The polymer according to any preceding claim, wherein the polymer is a homopolymer.

8. A polymer according to any preceding claim comprising the unit (II)

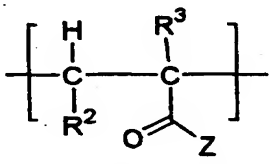


(II)

wherein R^2 is hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl, R^3 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl and isomers thereof, Z is a pendent group selected from the group consisting of $N(R^4)_2$, SR^5 and OR^6, wherein NR^4 is an aminoacyl group or N(oligopeptidyl group; R^6 and R^7 are selected from the group consisting of hydrogen, C_1C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy and C_1-C_{12} hydroxyalkyl, and may contain one or more cleavable bonds and may be covalently linked to a bioactive agent.

9. A polymer according to claim 8, wherein Z comprises one or more aminoacyl groups, preferably 2 to 6 aminoacyl groups, most preferably 4 aminoacyl groups.

10. A polymer according to claim 8 or 9 comprising the unit (II)



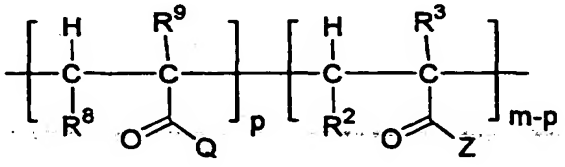
(II)

wherein R^2 is hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl, R^3 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl and isomers thereof, Z is a pendent group $N(R^4)_2$, wherein NR^4 is an aminoacyl group or N(oligopeptidyl group and wherein the polymer has a polydispersity of less than 1.2 and a molecular weight (Mw) of less than 50,000.

11. A polymer according to claim 8 to 10 wherein the bioactive agent is a drug.

12. A polymer according to claim 11 wherein the drug is an anti-cancer agent, preferably doxorubicin, daunomicin, taxol or paclaxitel.

13. A polymer according to any of claims 8 to 12, wherein the polymer has the structure (III)

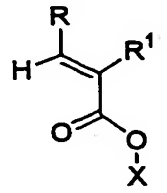


(III)

wherein R^8 and R^9 are selected from the same groups as R^2 and R^3 respectively, Q is a solubilising groups selected from the group consisting of C_1C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12} hydroxyalkyl, C_1-C_{12} alkylamido, C_1-C_{12} alkylimido, C_1-C_{12} alkanoyl, and wherein p is an integer of less than 500.

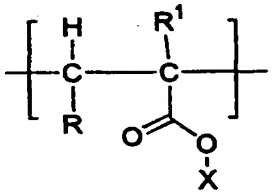
14. A polymer according to claim 13 wherein Q is a C_1-C_{12} hydroxyalkylamino group, preferably 2-hydroxypropylamino.

15. A process for the production of a polymer, comprising the radical polymerization of a compound (IV)



(IV)

wherein R is selected from hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl, is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl and isomers thereof; X is a carboxylate activating group; wherein the process is a controlled Radical Polymerization, to produce a polymer comprising the unit (I)



(I)

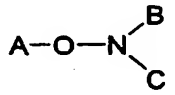
wherein n is an integer of 1 to 500.

16. A process according to claim 15, wherein the process is selected from the group consisting of Reversible Addition Chain Transfer Polymerization, Atom Transfer Radical Polymerization and Nitroxide Mediated Polymerization, preferably Atom Transfer Radical Polymerization.

17. The process according to claim 15 or 16, wherein the process additionally comprises a solvent and an Atom Transfer Radical Polymerization initiator which comprises a Cu(I)Br moiety complexed by a chelating ligand, preferably the initiator being $Cu(I)Br(Bipy)_2$ or Cu(I)Br (pentamethyldiethylene).

18. The process according to claim 17, wherein the solvent is an aprotic solvent selected from the group consisting of tetrahydrofuran, acetonitrile, dimethylformamide, acetone, dimethylsulphoxide, methylformamide and sulfolane.

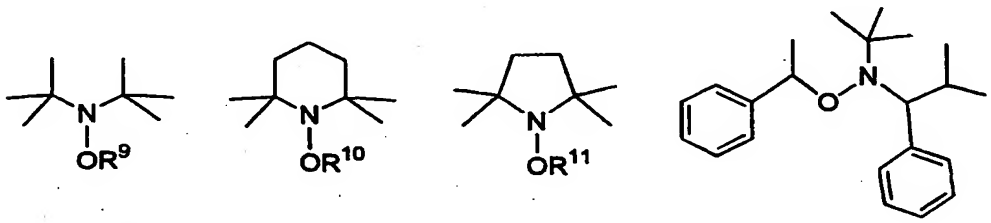
19. The process according to claim 16, wherein the polymerization is Nitroxide Mediate Polymerization that takes place in the presence of an initiator having the structure



wherein A is selected from the group consisting of C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} hydroxyalkyl, B and C are individually selected from the group consisting of C_1C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl and C_1-C_{12} hydroxyalkyl, may be joined so that together with N form a C_5-C_{12} heterocyclic group, and which may contain one or more additional heteroatoms selected from nitrogen, sulfur, oxygen and phosphorus.

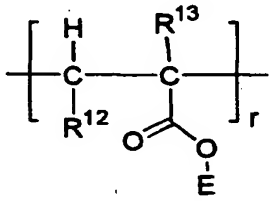
20. The process according to claim 19, wherein A is selected from the group consisting of methyl ethyl, propyl, butyl, pentyl, hexyl, benzyl, methylbenzene, ethyl benzene, propylbenzene or isomers thereof, and B and C are selected from the group consisting of isopropyl, isobutyl, secbutyl, tert-butyl, isopentyl, sec-pentyl, tert-pentyl, adamantyl, methylbenzene, ethyl benzene, propylbenzene or isomers thereof.

21. The process according to claim 19 wherein the initiator has a structure selected from the group consisting of



wherein R^9 to R^{11} are selected from the group consisting of C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl and C_1-C_{12} alkaryl.

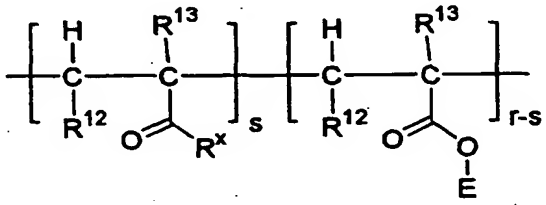
22. A process for the production of a polymer, comprising the reaction of a polymer having the formula (VI)



(VI)

wherein R^{12} is a group selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl and C_1-C_{18} alkaryl groups; R^{13} is selected from the group consisting of C_1-C_6 alkyl groups; E is a carboxylate

activating group and r is an integer of 5 to 500; with a reagent HR^x, wherein R^x is selected from the group consisting of $N(R^{14})_2$, SR^{15}, OR^{16}, wherein R^{14} to R^{17} are individually selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy and C_1-C_{12} hydroxyalkyl, and may contain one or more cleavable bonds, to form a derivatised polymer having the structure (VII)



(VII)

wherein $1 \leq s \leq r$.

23. A process according to claim 22 wherein R^{12} selected from the group consisting of hydrogen, methyl, ethyl and propyl, R^{13} is selected from the group consisting of hydrogen, methyl, ethyl and propyl and preferably R^{12} is hydrogen and R^{13} is methyl.

24. A process according to claim 22 or 23, wherein E is selected from the group consisting of N-succinimidyl, pentachlorophenyl, pentafluorophenyl, para-nitrophenyl, dinitrophenyl, N-phthalimido, N-norbornyl, cyanomethyl, pyridyl, trichlorotriazine, 5-chloroquinilino, and imidazole, preferably N-succinimidyl or imidazole, most preferably N-succinimidyl.

25. A process according to claim 23, wherein the polymer of formula (VI) is a polymer of formula (I) according to any of claims 1 to 7.

26. A process according to any of claims 15 to 21, wherein the polymer of the formula (I) is reacted in a second step with a reagent HR^x as defined in claim 22, whereby at least some of the groups -OX are replaced by $-R^x$ in the product derivatised polymer.

27. A process according to any of claims 22 to 26, wherein HR^x is $N(R^{14})_2$ preferably $N(R^{14})_2$ being an N-aminoacyl or N-oligopeptidyl group.

28. A process according to any of claims 22 to 27, wherein R^x comprises one or more aminoacyl groups, preferably 2 to 6, most preferably 4 aminoacyl groups.

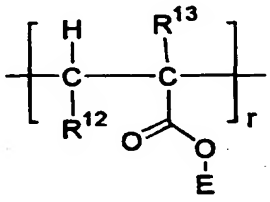
29. A process according to any of claims 22 to 28 wherein R^x comprises a bioactive agent, preferably an anti-cancer drug.

30. A process according to any of claims 22 to 29, comprising the additional step of reacting the unreacted groups, OE or OX groups, with a solubilising group selected from the group consisting of C_1-C_{12} alkyl, C_1-C_{12}

alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12} hydroxyalkyl, C_1-C_{12} alkylamido, C_1-C_{12} alkylimido, C_1-C_{12} alkanoyl.

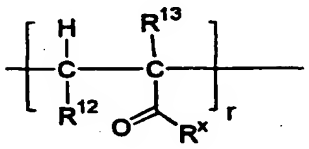
31. A process for the production of block copolymers comprising the steps of:

a. reacting a polymer having the formula (VI)



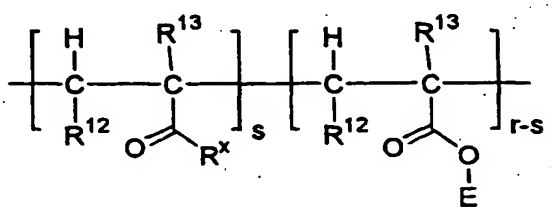
(VI)

wherein R^{12} is a group selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl and C_1-C_{18} alkaryl groups; R^{13} is selected from the group consisting of C_1-C_6 alkyl groups; E is a carboxylate activating group and r is an integer of 5 to 500; with a reagent HR^x, wherein R^x is selected from the group consisting of $N(R^{14})_2$, SR^{15}, OR^{16}, wherein R^{14} to R^{17} are individually selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy and C_1-C_{12} hydroxyalkyl, and may contain one or more cleavable bonds, to form a derivatised polymer having the structure (XV)



(XV)

b. reacting (XV) with a between 0.01 and 100 unit equivalents of (VI) to form a polymer (XVI)

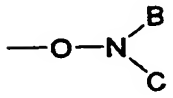


(VII)

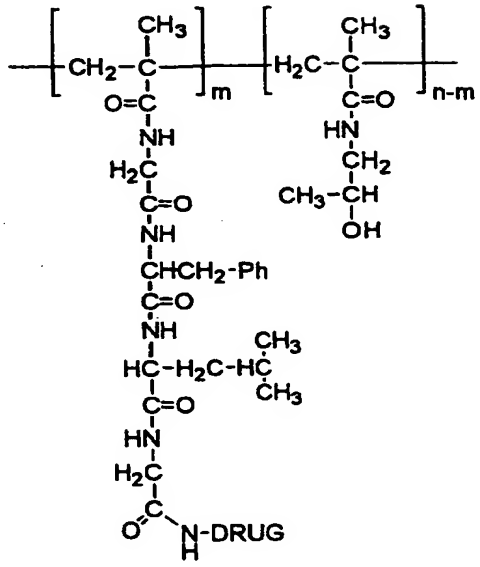
wherein $1 \leq s \leq r$.

32. A process according to claim 31 wherein (VII) is subsequently reacted with between 0.01 and 100 unit equivalents of reagent HR^x.

33. A process according to claim 31 or 32, wherein step B is a Controlled Radical Polymerisation process, preferably one in which polymer of the structure (XV) has one terminal group A and one terminal group

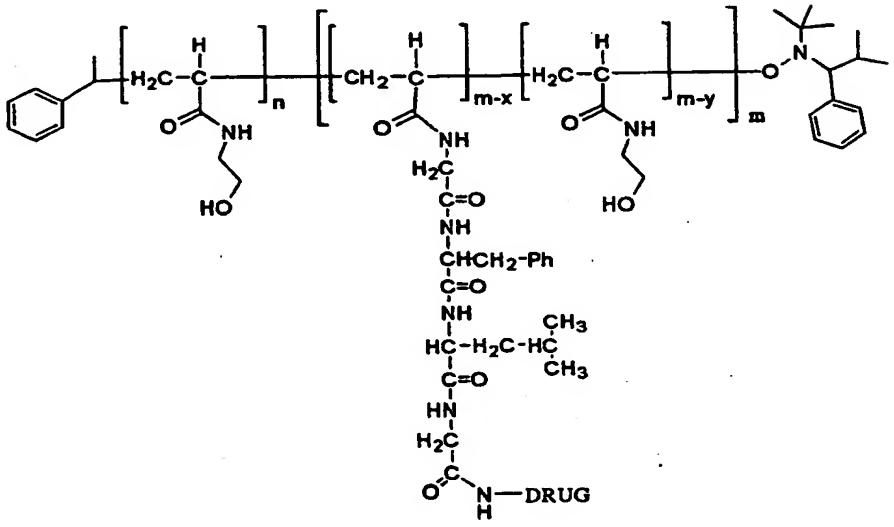


34. A polymer having the structure



wherein m is an integer of 1 to 500 and $1 \leq n \leq m$ and wherein the polymer has a polydispersity of less than 1.2.

35. A polymer having the structure



wherein m is an integer of 1 to 500 and $1 \leq n \leq m$ and $x+y=m$ and wherein the polymer has a polydispersity of less than 1.2.

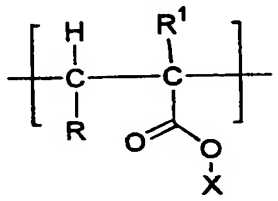
36. The polymer as defined in any of claims 1 to 14, 34 and 35 for use in a method of manufacture of a medicament, preferably for the treatment of cancer.

37. A composition comprising a polymer as defined in any of claims 1 to 14, 34 and 35 and a pharmaceutically acceptable excipient.

38. Use of a polymer as defined in any of claims 1 to 14, 34 and 35 as an excipient.

Abstract

A polymer comprising the unit (I)



(I)

where R is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl or any one of hydrogen, C_1-C_{18} alkyl, C_1-C_{18} alkenyl, C_1-C_{18} aralkyl, C_1-C_{18} alkaryl substituted with a heteroatom within, or attached to, the carbon backbone; R^1 is selected from the group consisting of C_1-C_6 alkyl groups; X is an carboxylate activating group and wherein the polymer has a polydispersity of less than 1.2 and a molecular weight (Mw) of less than 50,000, the polymer is preferably made by controlled radical polymerisation and is useful in the production of plymer drug conjugates with desirable biological profiles.

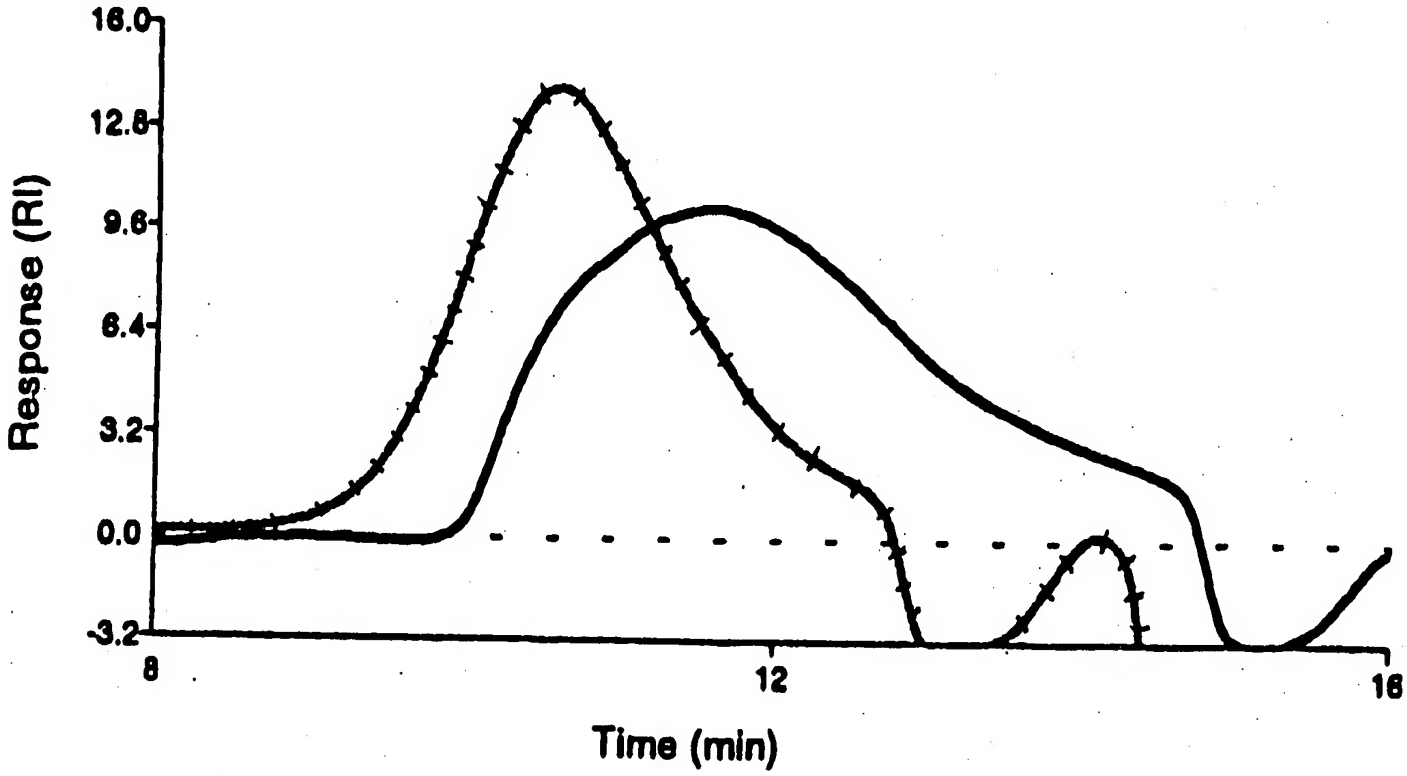


Figure 1